Low temperature and low moisture storage of seed of the endemic Australian genus *Eremophila* R Br (Myoporaceae)

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Abstract

Many species of the Australian endemic genus *Eremophila* (Myoporaceae) have the potential for minesite rehabilitation, revegetation and horticulture due to their demonstrated tolerance of fire, drought, salinity, frost and grazing. Fresh seed is not always available and so the ability to store seed, off site and under optimal conditions, without loss of viability, is an important land management option. Long term seed storage is an important *ex situ* conservation strategy for rare and threatened species. This study investigated the ability of *Eremophila* seed to survive desiccation to 5 ± 1% moisture content and storage at low temperature (–20 °C) for one year. Seed set in 14 species ranged from 1 to 91% and germination in 12 species ranged from 29 to 87% for fresh seed and 50 to 100% after storage for one year. Germination was lower in five collections after storage, although in three cases another cohort of the same species exhibited greater percent germination after storage. *Eremophila* seed has the potential for storage without loss of viability. Existing seed viability equations provide a framework for extrapolation of these short-term results to the long term that should offer predictions compatible with results after 125 years of storage.

Keywords: seed storage, *Eremophila*, low temperature, low moisture

Introduction

Eremophila are hardy perennial, prostrate to tall shrubs or small trees occurring throughout the arid- and semi-arid zones of mainland Australia. Their main centre of diversity is the Austin phytogeographic region of Western Australia (Beard 1980). They are an extremely versatile group of plants growing in a variety of soils including deep sands and gravelly clay loams, in both neutral and alkaline soils (Richmond 1993). *Eremophila* species are highly adapted to effective germination and growth under harsh conditions and in nutritionally impoverished soils if environmental conditions are favourable (Richmond 1993). Tolerance of these species to inhospitable environments such as dry, nutrient poor and saline sites (Elliott & Jones 1984) suggest that many species should be incorporated into minesite rehabilitation and rangeland revegetation programs (Richmond 1993). A number of species are highly ornamental, displaying large colourful flowers over an extended period of time, and have an outstanding potential for cultivation (Bond 1982; Richmond & Ghisalberti 1994a). *Eremophila* fruits are indehiscent and drupe-like, sometimes fleshy, with 2 to 4 locules per fruit (Chinnock 1981). One to three seeds may form per locule. Fruits may remain on plants for several months after ripening until dispersal by natural means or by animal or bird movement. In many species of *Eremophila* the woody endocarp structure of the fruit provides a physical (Richmond 1993) and possibly chemical (Warnes 1982; Richmond & Ghisalberti 1994b) barrier to germination. Plants are generally propagated vegetatively as germination from seed is unreliable (Elliot & Jones 1984; Richmond & Ghisalberti 1994b).

A number of studies have investigated various aspects of the taxonomy, biology, ecology and demography of the genus (*e.g.* Beard 1968; Barlow 1971; Smith 1975; Chinnock 1982; Richmond & Ghisalberti 1994a, 1996) and propagation of plants from seed (Richmond 1993; Richmond & Osborne 1993; Richmond & Chinnock 1994; Richmond & Ghisalberti 1994b). Natural regeneration of plants occurs after disturbance or heavy rains (Richmond & Ghisalberti 1996). No studies have been made on the storage potential of *Eremophila* seed, although Richmond & Ghisalberti (1994b) conducted research into the viability of *Eremophila* seed of different ages. There is some evidence to suggest loss of *Eremophila* seed viability with age (Elliott & Jones 1984; Richmond 1993).

It is widely accepted that viability of seed of many species may be lengthened under low temperature and low moisture conditions (Roberts 1973; Ellis *et al.* 1985; Dickie *et al.* 1990). International standards for long term storage of orthodox seed for genetic conservation recommend storage at moisture contents of 5 ± 1% (4% for oily seeds, 6% for starchy seeds), in hermetic containers at –18 °C or lower (Cromarty *et al.* 1990). Under these conditions, seed should remain viable for many hundreds of years.

Although species in the genus *Eremophila* have not previously been tested for response to low temperature and low moisture storage, a range of other Western Australian species have responded well to storage under

these conditions (Cochrane & Kelly 1996). The potential importance of members of the genus for rangeland revegetation, minesite rehabilitation and horticulture may rest on the ability of seed of those species remaining viable for long periods of time between collection and use in planting.

This study tests the hypothesis that seed of *Eremophila* species endemic to Western Australia are capable of desiccation and low temperature storage (–20 °C) without significant loss of viability. To test this hypothesis, an assessment of seed set and germinability was made for each species.

Methods

Fresh mature indehiscent fruits of 14 species of *Eremophila* were collected from 20 natural populations throughout south-western Western Australia between 1994 and 1999.

Seed Set

Seed set, defined in this study as the proportion of locules in a fruit containing at least one healthy seed, was determined by cutting woody fruits with a sharp scalpel under a dissecting microscope to establish the number of locules that contained whole seed. Fruit may be parthenocarpic and lacking evidence of seed initiation, or seed within fruits may be aborted, insect predated or dead (Richmond 1993). Fully developed seed are healthy, plump, firm and white.

Seed Germination and Storage

To overcome both the physical restraint and inhibitory nature of the fruit wall, and thus ensure successful germination, all seeds were excised from fruits prior to the establishment of germination trials. Extracted whole seeds were germinated on a 0.75% (w/v) agar solution in 90 mm glass Petri dishes in temperature and light controlled incubation cabinets, using a 12-hour photoperiod, at a constant 15 °C. The growth hormone gibberellic acid was added, as GA_3, to the agar solution at a concentration of 25 mg L^{-1} to assist with dormancy breaking. Previous trials on this genus have established that the addition of GA_3 offered higher percentage germination than seed germinated without treatment (A Cochrane, unpublished data).

Germination trials were undertaken on fresh seeds and on seeds after moisture content reduction and storage at –20 °C for a period of one year. All fruits were prepared for initial germination trials and storage within two months of collection. Seeds were stored within the woody fruits and whole fruits were dried for up to 8 weeks in a dehumidifying room at 15 °C and 15% relative humidity until moisture contents had reduced to 5 ± 1%. Seed moisture content was determined by the low constant temperature oven dry method (ISTA 1996). Fruits were hermetically sealed in laminated foil bags and frozen at –20 °C for storage.

After one year in storage a sample of fruits were unfrozen, allowed to re-hydrate for 24 hours to prevent imbibition damage, seed extracted and germination tests conducted. Pre- and post-storage germination treatments were identical. Petri dishes were checked twice weekly and germination was determined by radicle emergence.

Statistical Analysis

Cumulative germination percentages have been calculated on the basis of total seed numbers. Germination time has been expressed as the time in days from sowing to first germination and last germination. Arcsine transformed percentage values were analysed by one-way analysis of variance and Fisher's least significant differences. The probability level chosen for significant difference was $P < 0.05$. One-way analyses of variance were conducted on time to first and last germination.

Results

Seed Set

There was considerable spatial and temporal disparity in seed set in the genus, with demonstrated variation between species and between populations of the same species (Table 1). Reproductive success ranged from a low of 1% in one collection of *E. caerulea* subsp *merrallii* to

Table 1

Number of locules per fruit and mean seed set (percent of fruit with at least one locule containing seed) for 14 species of rare and threatened *Eremophila* from south-western Western Australia (n = number of fruit sectioned).

Species	Locules per fruit	% seed set Mean ± SE (range)	n	Collections tested
E. caerulea subsp *merrallii*	4	15 ± 5.8 (1-29)	154	4
E. chamaephila	3	37	65	1
E. lactea	2	48 ± 6.8 (33-65)	75	4
E. denticulata subsp *denticulata*	5+	80.5 ± 10.5 (70-91)	32	2
E. microtheca	4	53	100	1
E. nivea	4	57 ± 6.5 (32-75)	304	6
E. pinnatifida	2	27 ± 5.2 (14-41)	603	6
E. resinosa	4	31.5 ± 4.7 (24-45)	956	4
E. rostrata	4	32	110	1
E. scaberula	3	44 ± 15.6 (26-75)	300	3
E. subteretifolia	2	52.5 ± 22.5 (30-75)	30	2
E. veneta	4	60 ± 15.3 (30-80)	60	3
E. verticillata	2	18.75 ± 1.2 (17-22)	660	4
E. viscida	4	45.25 ± 9.2 (25-75)	80	4

Table 2

Percent germination for fresh and one year old stored seed for 12 species of *Eremophila* from 18 natural populations from south-western Western Australia.

Species	Population	Fresh Seed Germination (%)	Stored Seed Germination (%)
E. caerulea subsp *merrallii*	1	75	67
E. caerulea subsp *merrallii*	2	50	61
E. chamaephila	1	57	94
E. denticulata subsp *denticulata*	1	50	71
E. lactea	1	70	86
E. microtheca	1	73	76
E. nivea	1	67	50
E. nivea	2	80	100
E. nivea	3	63	83
E. pinnatifida	1	29	50
E. resinosa	1	77	67
E. scaberula	1	70	60
E. scaberula	2	39	67
E. scaberula	3	48	61
E. subteretifolia	1	83	62
E. veneta	1	38	60
E. veneta	2	38	100
E. viscida	1	87	90

91% in a collection of *E. denticulata* subsp *denticulata*. In many cases individual fruits contained no seed.

Seed Germination and Storage

The initial germination test results on fresh seed were variable, ranging from 29 to 87% (Table 2). Subsequent tests on stored seed showed less variability and ranged from 50 to 100%. The mean percent germination for fresh seed and for one year old stored seed was 61 ± 4% and 73 ± 3.7%.

Five collections (*E. caerulea* ssp *merrallii*, *E. nivea*, *E. resinosa*, *E. scaberula* and *E. subteretifolia*) failed to achieve the same or greater percent germination after storage. Three of these collections (*E. caerulea* ssp *merrallii*, *E. nivea* and *E. scaberula*) were represented by at least one other cohort of the same species from a different population that exhibited greater percent germination after storage indicating variation in germinability within the species. There was a significance difference between percent germination for fresh seed and for stored seed ($df=1$, $F = 4.45$, $P < 0.05$). The rate of germination differed between fresh and stored seeds (Table 3). There was a significant difference between overall time to first germination (10 ± 0.5 days for fresh seed *vs* 13 ± 1 days for stored seed) ($df = 1$, $F = 7.19$, $P < 0.01$) and last germination (24 ± 4 days for fresh seed *vs* 32 ± 4 days for stored seed) ($df = 1$, $F = 2.26$, $P < 0.01$).

Discussion

Bell *et al.* (1993) considered that low seed set in the genus *Eremophila* may be attributed to the ability of many species to resprout from protected buds after fire. All species investigated in this study are thought to regenerate from soil-stored seed reserve after fire yet fruits rarely contained the full complement of seed, with many empty. Resource limitations, in particular in populations that are located in fragmented landscapes, may contribute to this low reproductive success. Habitat fragmentation has the ability to change patterns of plant reproduction by affecting pollinators and predators, and the availability of potential mates, resources and microclimate. Cunningham (2000) attributed differences in fruit and seed production between different populations of *Eremophila glabra* to changes in important reproductive functions due to habitat fragment shape and size. In rare species that are restricted to small populations, the effects of inbreeding depression may impact on the reproductive success of outcrossing plants. The considerable inter- and intra-specific variation in seed set demonstrated in this study suggests the need for further investigation into both resource and genetic constraints in the genus *Eremophila*.

Despite low seed set for the species investigated in this study, germination was higher than previously noted for many species in *Eremophila*. The primary factor for poor germination from fruits of *Eremophila* is due to seed abortion (Richmond 1993) and the incubation of only healthy extracted seed in this study, rather than whole fruits, has no doubt led to these higher results.

These data suggest that seed of *Eremophila* have the ability to remain viable after drying and storage at sub-zero temperatures for periods of up to one year without a compromise in viability over the short term. The effects of moisture and temperature during storage are critical to the maintenance of seed viability. Extrapolation of results is required if data from short-term research is to provide advice on *ex situ* conservation of the species for the long term. A seed viability equation that provides a framework for extrapolation of these short term results to the long term should offer predictions compatible with results after 125 years of storage (Ellis & Roberts 1980).

Kullman (1981) reported the average time to germination of 28 species of *Eremophila* was 32.5 days under natural conditions, but did not provide seed age or quantities germinated or explain natural conditions. This present study has demonstrated a faster rate of

Table 3.

Germination times in days for fresh and one year old stored seed of 12 species of *Eremophila* from 18 natural populations from south-western Western Australia.

Species	Population	Fresh Seed		Stored Seed	
		Time to first germination	Time to last germination	Time to first germination	Time to last germination
E. caerulea subsp *merrallii*	BR	10	20	24	24
E. caerulea subsp *merrallii*	HR	12	54	14	39
E. chamaephila	GP	14	44	14	17
E. denticulata subsp *denticulata*	PR	8	8	9	24
E. lactea	GPW	12	12	11	39
E. microtheca	LI	11	15	9	45
E. nivea	BR	7	12	24	35
E. nivea	DR	7	21	8	11
E. nivea	KS	7	12	7	32
E. pinnatifida	D	11	19	14	74
E. resinosa	WR	10	74	14	35
E. scaberula	M20	9	16	9	48
E. scaberula	M5	10	17	14	21
E. scaberula	M9	8	18	14	38
E. subteretifolia	BL	9	33	14	28
E. veneta	HNR1	10	10	10	14
E. veneta	HNR2	10	14	10	14
E. viscida	ER	9	33	13	43

germination for a range of species under laboratory conditions than that reported by Richmond & Ghisalberti (1994b) for excised seed. This may be attributed to the additions of the growth hormone gibberellic acid, (as GA_3), which can promote germination in a range of species from Western Australia (Bell *et al.* 1993). The speed of germination in the field may be of importance for survival and successful establishment of plants. Whilst this research has demonstrated a slower rate of germination of seed post-storage, viability of that seed was not compromised. It is possible that the rate of germination of seed of these arid and semi-arid zone plants is inhibited by low temperature storage. Further investigations may be required to establish whether the success of field germination of stored seed is affected by low temperature and low moisture storage conditions.

For many species there will be years when seed set fails, and other years when seed yields are above average. Improving the potential for storage of seed means that collections of material can be made several years prior to their requirement in rehabilitation or revegetation programs, maximising the benefit of heavy yields that are usually a response to favourable environmental conditions. With so many species having tolerance to fire, drought, frost, grazing and salinity, the demand for seed material is unlikely to abate with future increases predicted.

Seed storage by conventional means (low moisture and low temperature) offers a cheap and effective method of preserving a broad range of genetic material for short term rehabilitation and revegetation needs, and goes a long way to meeting the long term challenge of *ex situ* conservation of endangered flora in Western Australia. In addition to the requirements for minesite rehabilitation for widespread species, there are some 71 rare, threatened and poorly known species of *Eremophila* endemic to Western Australia that will benefit from the advances made in the identification of appropriate storage conditions for maximising long term viability in the genus.

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